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Acute Toxicity of Nitrite to Rainbow Trout (Salmo gairdneri): Effects of pH, Nitrite Species, and Anion Species

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Acute Toxicity of Nitrite to Rainbow Trout (*Salmo gairdneri*): Effects of pH, Nitrite Species, and Anion Species

ROSEMARIE C. RUSSO, ROBERT V. THURSTON, AND KENNETH EMERSON

Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT 59717, USA

RUSSO, R. C., R. V. THURSTON, AND K. EMERSON. 1981. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species, and anion species. Can. J. Fish. Aquat. Sci. 38: 387–393.

The toxicity of nitrite to rainbow trout is pH-dependent within the range considered acceptable to most freshwater aquatic life (pH 6.5–9.0). Both of the nitrite species, NO_2^- and HNO_2, are toxic. It is recommended that nitrite criteria to protect freshwater aquatic life be based on total nitrite, and that such criteria reflect the pH dependence of nitrite toxicity. Variation in the toxicity of nitrite in the presence of chloride, sulfate, phosphate, and nitrate anions has also been demonstrated. It is concluded that the toxicity of nitrite to fishes, in addition to being pH-dependent, is also dependent in varying degrees upon many of the anions that are commonly found in natural aquatic environments.

Key words: nitrite, pH, chloride, phosphate, sulfate, toxicity, rainbow trout, *Salmo gairdneri*

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La toxicité des nitrites à l'endroit de la truite arc-en-ciel dépend du pH dans la gamme généralement considérée acceptable à la plupart des organismes aquatiques (pH 6,5–9,0). Les deux espèces de nitrites, NO_2^- et HNO_2, sont toxiques. Les critères de nitrites établis en vue de protéger la vie dans les eaux douces devraient être fondés sur les nitrites totaux, et ces critères devraient refléter l'influence du pH sur leur toxicité. On a également démontré des variations de toxicité en présence des anions chlorure, sulfate, phosphate et nitrate. Nous concluons que la toxicité des nitrites à l'endroit de poissons, en plus de dépendre du pH, est également influencée à divers degrés par plusieurs anions communément rencontrés dans les milieux aquatiques naturels.

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NITRITE has received increasing attention over the past few years as a potentially important pollutant in aquatic systems. Streams receiving effluent from sewage treatment plants may be subjected to elevated concentrations of nitrite due to an imbalance in the nitrification process. Nitrite can also be a problem at fish culture facilities which have water reuse systems using the nitrification process for removing ammonia. Nitrite in very low concentrations is known to be toxic to fishes, but only recently have researchers begun to study the importance of the effect of water chemistry conditions on nitrite toxicity. The presence of chloride ion inhibits nitrite toxicity (Russo and Thurston 1977; Perrone and Meade 1977; Tomasso et al. 1979), as does calcium (Crawford and Allen 1977). A reduction in nitrite toxicity as pH increases has been reported by Wedemeyer and Yasutake (1978); these authors tested steelhead trout (*Salmo gairdneri*) in static bioassays at pH 6, 7, and 8. Published information on nitrite toxicity to fishes has been summarized by Russo and Thurston (1977), U.S. EPA (1977), and Patrick et al. (1979).

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In aqueous solution, nitrite establishes the following equilibrium:

$$NO_2^- + H^+ \rightleftarrows HNO_{2(aq)}.$$

This equilibrium is affected by pH, and toxicity might therefore also be affected by pH. The present study was initiated to investigate the effect of pH on nitrite toxicity to fishes, to examine whether different acids used for pH reduction exhibited different effects on such toxicity, and to determine whether nitrite ion itself or the free acid HNO_2 is the principal toxic species. The effect of chloride on nitrite toxicity at different pH levels was also investigated.

Methods

Four series of 96-h bioassays were conducted using rainbow trout (*Salmo gairdneri*). Series I (12 tests) and series II (22 tests) were conducted over the pH range 6.4–9.0; this range was selected because a pH criterion of 6.5–9.0 has been recommended by the U.S. Environmental Protection Agency (1977) for the protection of freshwater aquatic life.

The two series were conducted on two different size ranges of fish and in two different years. Series III (six tests) was conducted at pH 7, using three different acids (H_2SO_4, H_3PO_4, and HNO_3) for pH reduction. Series IV (four tests) was conducted over the pH range 7.5–8.6, at chloride concentrations above background.

Tests were conducted in either circular fiberglass tanks (diameter 1.2 m) with a water volume of 350 L or in copolymer plastic tanks with a water volume of 62 L. One control and five test tanks were used for each test. Dilution water was obtained from a ground spring source and had the chemical characteristics shown in Table 1. All test chemicals added were reagent grade. Concentrated sodium nitrite ($NaNO_2$) solution was added to the 350-L tanks from a single source by means of fixed-speed metering pumps (Masterflex, Barnant Corp.), but at a different flow rate to each tank to achieve the desired concentration gradient differences among the tanks. The toxicant solution was added at the point of addition of dilution water to ensure complete mixing; spot analyses confirmed that mixing was complete and immediate. Water replacement time in the tanks was ~1.3 h. Toxicant and dilution water added to the 62-L tanks was by means of an intermittent flow system using proportional diluters of the basic design of Mount and Brungs (1967) with a flow rate of 500 mL every 2.5–3 min; water replacement time was ~5 h.

The pH levels desired were achieved by adding dilute aqueous solutions of either sodium hydroxide (NaOH) to raise the pH, or phosphoric acid (H_3PO_4), sulfuric acid (H_2SO_4), nitric acid (HNO_3), or hydrochloric acid (HCl) to lower the pH. The acid and base solutions were added by means of variable-speed metering pumps (Fluid Metering, Inc.) as was sodium chloride (NaCl) solution for the series IV tests.

Test fish were hatchery-reared rainbow trout obtained from the Fish Cultural Development Center, U.S. Fish and Wildlife Service (F&WS), Bozeman, Montana; fish were cultured in water from the same source as that used for test dilution water. Fish were collectively weighed before the test and randomly distributed among the test tanks, 10 or 20 fish to each tank for all of the tests except three of the tests in series I in which five fish were used in each tank. Fish were fed a commercial salmon ration (Sterling Nelson & Sons, Murray, Utah) twice daily prior to testing; they were not fed during the acclimation and test periods. Fish were acclimated to the test pH by gradually adjusting the dilution water pH, over a 24-h period, to the desired value and maintaining that value for 48 h before addition of the toxicant. Fish mortality observations were made and dead fish removed at 4- to 8-h intervals during the first 24 h of each test, then at 6- to 12-h intervals during the next 72 h; fish mortality was evidenced by complete immobilization and lack of respiration. Fish lengths (total length) were measured as dead fish were removed, and survivors' lengths were measured at the termination of each test. There was one control death (10%) in each of tests 607 and 609; no control fish died during any of the other tests.

The water in each test tank was analyzed for nitrite three times during each test, and pH was determined 2–3 times daily; fewer measurements were made in those tanks in which all fish died during the first 72 h. Other water chemistry variables were measured 1–3 times in each tank during each test. Total nitrite-nitrogen was measured by the sulfanilamide–naphthylethylenediamine method (U.S. EPA 1974), and carbon dioxide by H_2SO_4 titration (ASTM 1976). Temperature was measured with a calibrated mercury thermometer, and pH with a digital meter (Beckman Phasar-I). All other analytical procedures were according to APHA et al. (1976): nitrate-nitrogen, ultraviolet spectrophotometric method, corrected for nitrite interference; ammonia-nitrogen, nesslerization method; dissolved oxygen, either with a meter (Yellow Springs Instrument model 54) or by the azide modification of the iodometric titration method with phenylarsine oxide substituted for sodium thiosulfate; total alkalinity, H_2SO_4 titration; total hardness and calcium, both by EDTA titration; chloride, $Hg(NO_3)_2$ titration. All colorimetric measurements were made using a Varian model 635 ultraviolet-visible spectrophotometer. Averaging of pH was accomplished using a computer program which converts pH values to their corresponding hydrogen ion concentrations, averages these, then reconverts the average to pH units.

TABLE 1. Chemical characteristics of the dilution water used in bioassays. (All values are mg/L unless otherwise noted.)

Al	<1	Ni	<0.005
As	0.0012	Pb	<0.015
Cd	<0.005	Se	0.00085
Cr	<0.005	Zn	0.01
Cu	0.007	F^-	0.35
Fe	0.004	PO_4^{3-}	0.05
Hg	<0.00005	SO_4^{2-}	17.2
K	0.82	Total organic carbon	3.3
Mg	16.7	SEC, μsiemens 25°C	340
Mn	0.002	Turbidity, NTU	1.6
Na	2.5		

Ranges for the variables temperature, dissolved oxygen, hardness, nitrate, total ammonia, chloride, and calcium are given in the tables of results. None of these, except in those tests in which nitrate or chloride was purposely added, was considered to be a variable which affected the experimental results. Alkalinity for each test is listed in the tables, and concentration of acid anion is given for the series III tests. Carbon dioxide concentrations were determined separately for the series II and III tests by the bicarbonate titration method (ASTM 1976); the values obtained were in good agreement with the measured values of alkalinity, as would be expected. Water chemistry data for the individual tests are available upon request.

Median lethal concentration (LC50) values and their 95% confidence interval endpoints were calculated using a computer program developed for the Trimmed Spearman–Karber Method (Hamilton et al. 1977); the calculations were performed using an XDS Sigma 7 computer.

The molar concentration of nitrous acid, HNO_2, was calculated using the equation

$$\log[HNO_2] = \log[NO_2^-] + pK_a - pH$$

where $[NO_2^-]$ was the measured nitrite concentration (converted to mol/L) and pH was the measured experimental pH. A value of 3.39 ± 0.03 was used for pK_a; this constant has not been accurately measured as a function of temperature, but represents a best estimate of its value, based on a plot

TABLE 2. Acute toxicity of nitrite at different pH values to rainbow trout (series I).[a]

Test No.	Average (mean) fish size		Fish per tank	Acid or base	Alkalinity mg/L $CaCO_3$ (range)	pH (range)	96-h LC50	
	Wt (g)	Length (cm)					mg/L NO_2-N (95% C.I.)	ng/L HNO_2-N (95% C.I.)
489	285	28.1	5	H_3PO_4	109 (108–113)	6.44 (6.28–6.56)	0.21 (0.13–0.34)	195 (119–319)
417	171	23.2	10	H_3PO_4	171 (170–171)	7.50 (7.47–7.53)	0.20 (0.17–0.24)	16.0 (13.5–18.8)
344	46.7	15.3	20	H_3PO_4	174 (172–175)	7.52 (7.44–7.63)	0.32 (0.26–0.39)	24.2 (20.2–29.1)
243	53.1	15.7	20	None	176 (175–177)	7.68 (7.58–7.79)	0.27 (0.22–0.32)	13.8 (11.3–16.8)
505	387	29.7	5	None	159 (158–160)	7.74 (7.69–7.80)	0.24 (0.17–0.33)	10.8 (7.75–15.2)
244	60.5	16.6	20	None	177 (175–180)	7.76 (7.71–7.83)	0.27 (0.23–0.32)	11.4 (9.8–13.3)
423	188	23.6	10	None	172 (171–172)	7.81 (7.76–7.86)	0.19 (0.15–0.24)	7.57 (6.02–9.51)
326	24.3	12.3	20	None	179 (178–180)	8.10 (7.99–8.33)	0.28 (0.24–0.32)	5.43 (4.72–6.25)
384	121	20.5	10	NaOH	176 (175–176)	8.15 (7.94–8.60)	0.41 (0.32–0.52)	7.65 (6.17–9.49)
248	74.8	17.4	10	NaOH	180 (179–182)	8.23 (8.07–8.63)	0.38 (0.31–0.46)	5.34 (4.40–6.48)
247	69.8	17.0	10	NaOH	182 (180–183)	8.24 (8.13–8.55)	0.39 (0.33–0.47)	5.35 (4.48–6.39)
500	341	28.4	5	NaOH	190 (189–192)	9.00 (8.97–9.05)	1.67 (1.04–2.70)	4.07 (2.45–6.77)

[a]The range of other water chemistry variables (in mg/L) for all tanks at time of tests were dissolved oxygen 7.6–9.3; NH_3-N 0.00–0.11 (test 417 0.06–0.15); Cl^- 0.00–1.71; Ca^{2+} 54.9–65.7; hardness (as $CaCO_3$) 185–208. Temperature range for all tanks was 9.0–10.7°C.

of the data compiled by Sillén and Martell (1964). A temperature of 10°C was used to estimate the value of pK_a. Temperatures in the bioassays conducted ranged from 9 to 14°C, and differences were considered to be insignificant in determination of the results. Changes in the value of the constant will change the absolute values of LC50s for nitrous acid reported here, but will not change any of the trends upon which our conclusions are based.

LC50 values in terms of total nitrite-nitrogen (NO_2-N) were calculated using measured total NO_2-N concentrations and mortality observations. For calculation of LC50 values in terms of nitrous acid-nitrogen (HNO_2-N) concentrations, a computer program was written which calculated the HNO_2-N concentration in ng/L for each test tank in a given bioassay from the measured NO_2-N concentration in mg/L and pH. Using these HNO_2-N values in combination with the fish mortality data for each time of observation, the LC50 was then recalculated in terms of HNO_2-N concentration.

Results and Discussion

Effect of pH and Nitrite Species

Ninety-six-hour LC50 values in terms of HNO_2 and NO_2^- (both expressed in terms of N) for the series I and series II experiments are reported in Tables 2 and 3. The results of both series are similar. If all toxicity resided in either nitrite species, independent of pH, a plot of LC50 values for that species would be a horizontal line. It is apparent that as pH increases, toxicity in terms of NO_2-N decreases (Fig. 1), and toxicity in terms of HNO_2-N increases (Fig. 2).

The simplest explanation for the shapes of these two plots is that neither species alone is responsible for the entire toxicity. Both must contribute, though not necessarily equally. If the toxicity of NO_2^- were insignificant compared to that of HNO_2 at the lower end of the pH range studied, then the LC50 curve for HNO_2 would plateau at low pH. Such is not the case. Neither is it the case that the NO_2^- LC50 curve plateaus at the upper end of the pH range studied. It is concluded that within the entire pH range both species contribute significantly to the total toxicity. Because HNO_2 is present in amounts ranging from about one part in 10^4–10^6 of total nitrite, it is further concluded that its toxicity must be higher per unit weight than that of the ion.

It has been suggested that the toxicity of nitrite may depend upon the concentration of HNO_2 rather than NO_2^- because the free acid can move more readily across biological membranes than can its ionic form (Colt and Tchobanoglous 1976). Our data lead us to the conclusion, however, that NO_2^- contributes significantly to the total toxicity. Evaluation of our hypothesis necessitates a clearer understanding of the uptake by fishes of both the NO_2^- and HNO_2 species.

Comparative Effect of Sulfate, Phosphate, and Nitrate Ions

To determine what might be the effect of using an acid other than H_2SO_4 to reduce pH in the test water, thus adding

TABLE 3. Acute toxicity of nitrite at different pH values to rainbow trout (series II).[b] (Ten fish per tank in all tests.)

Test No.	Average (mean) fish size		Acid or base	Alkalinity mg/L $CaCO_3$ (range)	pH (range)	96-h LC50	
	Wt (g)	Length (cm)				mg/L NO_2-N (95% C.I.)	ng/L HNO_2-N
603	12.8	10.4	H_2SO_4	120 (117–123)	6.99 (6.84–7.04)	0.14 (0.12–0 15)	34.4 (30.6–38.8)
601	15.3	10.2	H_2SO_4	128 (124–130)	7.02 (6.96–7.07)	0.11 (0.092–0.13)	25.9 (21.9–30.7)
598	10.4	10.0	H_2SO_4	135 (128–142)	7.24 (7.16–7.33)	0.15 (0.13–0.18)	21.6 (18.4–25.3)
595	10.0	9.4	H_2SO_4	150 (146–154)	7.37 (7.32–7.41)	0.19 —[b]	19.4 —
593	9.3	9.2	H_2SO_4	160 (158–164)	7.59 (7.53–7.64)	0.18 (0.15–0.21)	10.8 (9.25–12.6)
585	7.0	9.1	None	188 (188–189)	7.83 (7.75–7.91)	0.40 —	13.6 —
605	12.8	10.3	None	173 (172–176)	7.86 (7.81–7.93)	0.21 (0.19–0.24)	7.19 (6.47–7.98)
610	13.2	10.3	None	177 (174–180)	7.87 (7.82–7.92)	0.22 (0.18–0.27)	7.26 (6.07–8.68)
587	8.0	8.6	None	170 (168–170)	7.88 (7.77–7.99)	0.36 (0.33–0.39)	11.4 (10.6–12.3)
597	10.0	9.3	None	172 (170–174)	7.90 (7.84–7.94)	0.21 (0.19–0.24)	6.55 (5.87–7.32)
590	8.2	8.7	None	170 (168–171)	7.91 (7.82–8.01)	0.30 (0.26–0.34)	9.05 (8.09–10.1)
579	3.1	6.3	None	165 (164–166)	7.94 (7.91–8.00)	0.25 (0.21–0.30)	7.35 (6.14–8.81)
600	10.4	9.2	None	174 (172–176)	7.95 (7.86–8.09)	0.17 (0.15–0.20)	4.88 (4.22–5.66)
592	8.4	8.9	NaOH	178 (177–179)	8.27 8.21–8.36)	0.54 (0.50–0.60)	7.19 (6.58–7.86)
604	12.8	10.2	NaOH	185 (183–186)	8.41 (8.32–8.60)	0.46 (0.40–0.53)	4.34 (3.84–4.91)
607	15.5	10.9	NaOH	188 (186–190)	8.49 (8.33–8.59)	0.45 (0.39–0.52)	3.64 (3.14–4.23)
602	15.3	10.0	NaOH	186 (185–188)	8.61 (8.53–8.74)	0.50 (0.44–0.57)	3.02 (2.71–3.37)
589	8.2	8.8	NaOH	181 (180–182)	8.70 (8.64–8.78)	0.71 (0.63–0.80)	3.48 (3.10–3.91)
594	9.3	9.4	NaOH	188 (187–190)	8.83 (8.77–8.90)	1.17 (0.82–1.67)	4.30 (2.98–6.20)
599	10.4	9.6	NaOH	191 (190–193)	8.83 (8.75–8.91)	0.61 (0.52–0.71)	2.23 (1.94–2.57)
584	7.0	8.7	NaOH	202 (201–203)	9.03 (8.94–9.09)	1.10 —	2.35 —
586	8.0	8.8	NaOH	190 (188–192)	9.04 (8.97–9.12)	1.12 (0.91–1.37)	2.47 (1.99–3.06)

[a]The range of other water chemistry variables (in mg/L) for all tanks at time of tests were dissolved oxygen 6.6–9.3; NH_3-N 0.00–0.06; Cl^- 0.00–0.47; Ca^{2+} 46.8–61.3; hardness (as $CaCO_3$) 178–209. Temperature range for all tanks was 8.9–14.7°C.

[b]Confidence intervals not calculable from statistical method.

different anions, four additional tests were conducted at pH 7, two of these using H_3PO_4 and two using HNO_3. The fish tested were from the same pool as that from which fish were taken for the series II tests in which H_2SO_4 had been used. In that series, two tests had been conducted at pH 7, and these two tests were used for comparison with the H_3PO_4 and HNO_3 tests, also conducted at pH 7. The results of all six of these tests (series III), are reported in Table 4.

Variation in average pH among these six tests was minimal (±0.02 pH unit), and replicability of LC50 results in any pair of tests was extremely good. The LC50 values obtained, in terms of both NO_2-N and HNO_2-N, were lowest in the presence of sulfate and highest in the presence of nitrate.

EFFECT OF CHLORIDE ION

The suppression of nitrite toxicity in the presence of chloride ion has been reported previously for rainbow trout (Russo

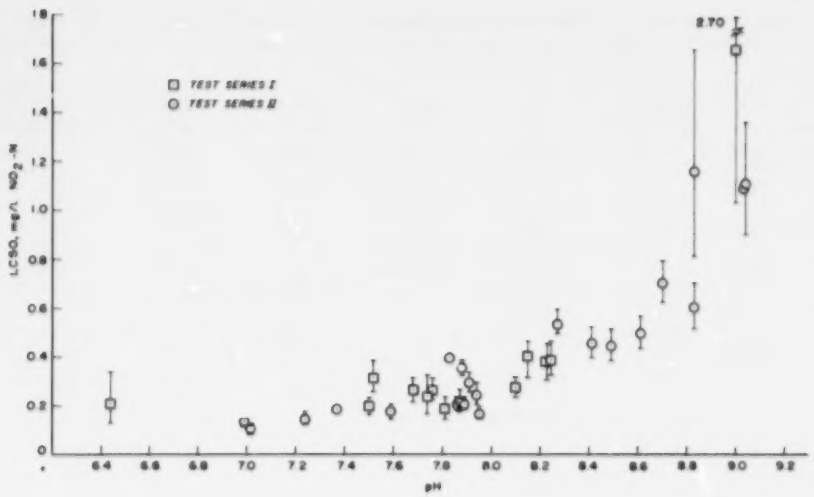

FIG. 1. Acute toxicity to rainbow trout of nitrite over the pH range 6.4–9.0, LC50 as NO_2-N vs. pH. (Error bars are 95% confidence intervals.)

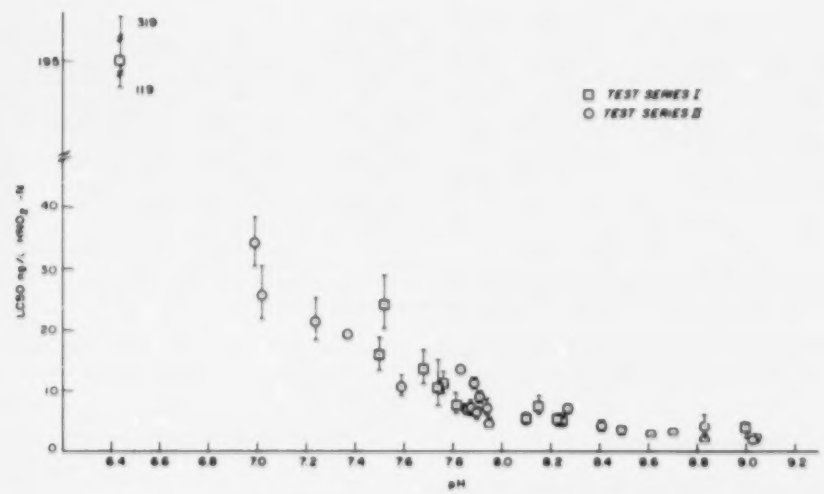

FIG. 2. Acute toxicity to rainbow trout of nitrite over the pH range 6.4–9.0, LC50 as HNO_2-N vs. pH. (Error bars are 95% confidence intervals.)

and Thurston 1977), coho salmon (*Oncorhynchus kisutch*) (Perrone and Meade 1977), steelhead trout (Wedemeyer and Yasutake 1978), and channel catfish (*Ictalurus punctatus*) (Tomasso et al. 1979). Additional data in support of these earlier findings are provided by the results of the series IV experiments (Table 5). These bioassays were conducted at a chloride concentration of ~10 mg/L and over the pH range 7.5–8.6.

The 96-h LC50 values were an order of magnitude higher than, and followed the same trend as, those obtained for the fish tested over the same pH range without chloride addition (series I and II). The NO_2-N values with chloride added were 3.5–5.3 mg/L, and without they were 0.17–0.54 mg/L. The HNO_2-N values with chloride added were 280–34 ng/L, and without they were 24–3.0 ng/L.

Fish size varied from 28 to 244 g in the chloride-added experiments, but this was not considered to be a factor in results. Russo (1980) has reported that under similar test and

TABLE 4. Acute toxicity of nitrite at pH 7 to rainbow trout, using different acids for pH control (series III).[a] (Ten fish per tank in all tests.)

Test No.	Average (mean) fish size Wt. (g)	Length (cm)	Acid	Alkalinity mg/L $CaCO_3$ (range)	pH (range)	SO_4^{2-} mg/L (range)	PO_4-P mg/L (range)	NO_3-N mg/L (range)	96-h LC50 mg/L NO_2-N (95% C.I.)	96-h LC50 ng/L HNO_2-N (95% C.I.)
601	15.3	10.2	H_2SO_4	128 (124–130)	7.02 (6.96–7.07)	74.0 (70.8–77.2)	0.05	0.14 (0.12–0.15)	0.11 (0.092–0.13)	25.9 (21.9–30.7)
603	12.8	10.4	H_2SO_4	120 (117–123)	6.99 (6.84–7.04)	79.3 (77.6–80.8)	0.05	0.13 (0.12–0.14)	0.14 (0.12–0.15)	34.4 (30.6–38.8)
609	13.2	10.7	H_3PO_4	152 (151–153)	6.99 (6.95–7.06)	17.2	18.2 (17.8–18.9)	0.13 (0.12–0.13)	0.18 (0.13–0.26)	46.1 (33.8–63.0)
612	13.7	10.8	H_3PO_4	150 (146–155)	6.98 (6.95–7.04)	17.2	19.1 (16.6–20.6)	0.10 (0.09–0.12)	0.18 (0.16–0.21)	48.2 (42.5–54.8)
611	13.7	10.6	HNO_3	137 (130–144)	7.01 (6.95–7.08)	17.2	0.05	9.86 (6.76–12.4)	0.29 (0.26–0.32)	67.6 (59.7–76.7)
613	18.2	11.8	HNO_3	136 (131–140)	7.01 (6.95–7.16)	17.2	0.05	11.5 (10.3–13.0)	0.26 (0.23–0.29)	59.2 (54.0–65.0)

[a]Sulfate and phosphate values reported without range were not measured during tests; values reported are those for dilution water. The range of other water chemistry variables (in mg/L) for all tanks at time of tests were dissolved oxygen 6.6–6.8; NH_3-N 0.00–0.06; Cl^- 0.10–0.63; Ca^{2+} 48.9–52.9; hardness (as $CaCO_3$) 192–201. Temperature range for all tanks was 9.4–14.0°C.

TABLE 5. Acute toxicity of nitrite at 10 mg/L chloride over different pH values to rainbow trout (series IV).[a] (Twenty fish per tank in test 329; all other tests 10 fish per tank.)

Test No.	Average (mean) fish size Wt. (g)	Length (cm)	Chloride mg/L (range)	Acid or base	Alkalinity mg/L $CaCO_3$ (range)	pH (range)	96-h LC50 mg/L NO_2-N (95% C.I.)	96-h LC50 ng/L HNO_2-N (95% C.I.)
329	28.2	12.9	10.9 (8.5–13.9)	HCl	174 (173–176)	7.50 (7.38–7.61)	3.74 (3.25–4.30)	284 (246–328)
363	79.0	17.6	10.4 (9.2–13.0)	None	177 (176–178)	7.90 (7.83–7.96)	3.54 (2.96–4.22)	109 (91.5–130)
401	147	22.0	10.6 (10.1–12.2)	NaOH	188 (186–190)	8.47 (8.39–8.58)	4.35 (3.60–5.26)	36.3 (30.2–43.5)
460	244	26.1	10.5 (9.8–12.5)	NaOH	184 —	8.59 (8.50–8.67)	5.34 (4.43–6.45)	34.3 (28.8–40.9)

[a]The range of other water chemistry variables (in mg/L) for all tanks at time of tests were dissolved oxygen 7.2–9.6; NH_3-N 0.00–0.06; hardness (as $CaCO_3$) 199–206. Temperature range for all tanks was 9.9–10.7°C.

uniform water conditions, there was no statistically significant difference in nitrite toxicity to rainbow trout ranging in size from 2 to 387 g.

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